BACTERIAL LEAF SPOT OF UMBELLULARIA CALIFORNICA[1]

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Diseased leaves were collected from a California laurel tree, *Umbellularia californica* Nuttall, located on the west slope of the Berkeley hills just east of the University of California campus. Plants growing in this area are subjected to relatively high moisture conditions due to the coastal fogs which prevail through several months of the year. Leaves of *U. californica* were also collected extensively in other coastal areas of California from Santa Cruz to Mendocino counties. The disease under discussion, however, was found only on trees in the Berkeley area.

SYMPTOMS

Black, angular leaf spots, delimited more or less by the small veinlets in the leaves, are the symptoms of this disease (fig. 1). In old infections the central part of the spot dies and the area around the periphery of the spot often yellows. Necrotic spots often contain swollen portions which, when pierced by a dissecting needle, yield a white bacterial ooze. The disease appears to be associated with wounds since it is often found in nature on leaves which have been scratched due to wind movement; in artificial inoculations only occasional infections were induced on unwounded leaves. When the leaves are extensively wounded and inoculated, infection is very severe, the spots become confluent and eventually the whole leaf is killed.

ISOLATION OF THE CAUSAL AGENT AND INOCULATION OF THE HOST

The diseased areas of leaves were cut out and surface sterilized by dipping in 95 per cent ethyl alcohol for one second, after which they were placed in a 1-1000 solution of mercuric chloride for one minute according to the method outlined by Elliott (1920). The diseased portions of leaves were then washed successively in ten sterile water blanks and placed in a dry, sterile petri dish to drain. Sterile instruments were used to cut out and place the individual diseased spots of the leaf on potato dextrose agar plates.

After forty-eight hours of culturing bacteria grew out onto the medium from the leaf sections thus prepared.

[1] A revision of a section of a thesis submitted to the Graduate Division of the University of California at Berkeley in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to acknowledge his indebtedness to Dr. H. N. Hansen and other members of the Division of Plant Pathology for counsel and advice in this work.

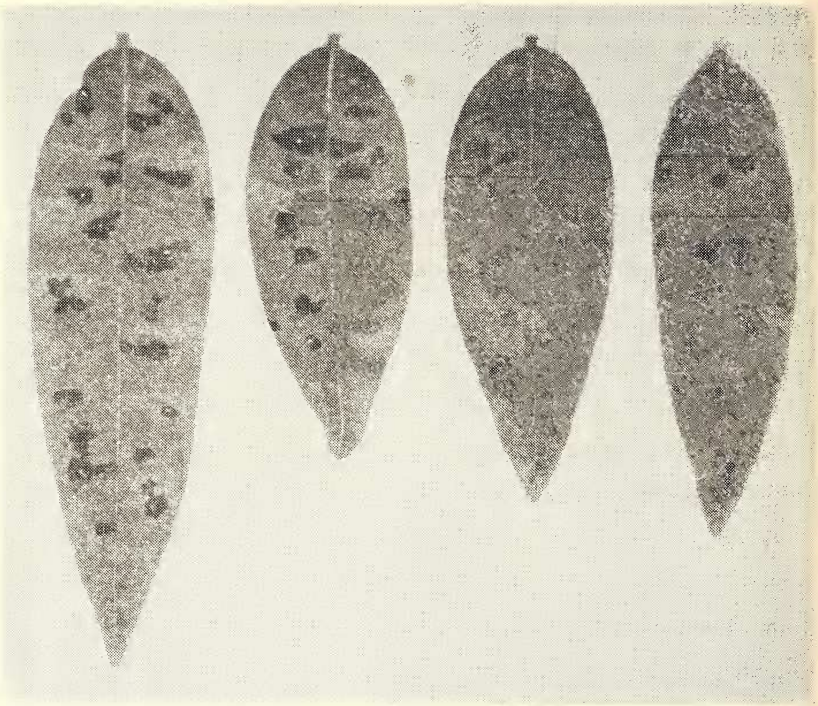

FIG. 1. Infected leaves of *Umbellularia californica* after artificial inoculation with *Pseudomonas lauracearum*.

A small amount of this inoculum was transferred to a sterile water blank and from this bacterial suspension streaks were made on potato dextrose plates. Streaks made from the resultant colonies produced pure cultures of the pathogen which were maintained on potato dextrose slants.

The work of Johnson (1947) suggests the value of predisposing plants to infection by means of water congestion. Accordingly, one year-old potted seedlings of *U. californica* were placed in a moist chamber out of doors in Berkeley, California, for forty-eight hours. Such a moist environment is often approximated in the foggy area where the disease was observed.

Two leaves on each seedling were then wounded by puncturing with a dissecting needle, two by scratching with a dissecting needle and the remainder were left unwounded. These seedlings were sprayed with a suspension of the bacterium isolated from diseased *U. californica* leaves and were returned to the moist chamber for an additional seventy-two hours. Thereupon the plants were removed and placed in a shaded location out of doors. Seven days after inoculation, black spots were visible on the punctured and scratched

leaves and on the latter the spots soon became confluent, the infection spreading over the whole leaf. Immature leaves did not become infected indicating that entrance of the pathogen is probably not through the stomata. Infection occurred rarely on unwounded leaves. However, in a few of the older, unwounded leaves, lesions occasionally appeared near the tips and lateral margins, pointing to the possible entry of bacteria through hydathodes.

The bacterium was reisolated from these artificially induced lesions by the same method as used for the original isolation. Subsequent inoculations made with this reisolate produced typical symptoms.

Seedlings of orange (*Citrus sinensis* Osbeck), Pinto bean (*Phaseolus vulgaris* L.) and avocado (*Persea drymifolia* Cham. & Schlecht.) were inoculated in the same manner as were the *Umbellularia californica* seedlings. Avocado was the only one of these plants that became infected. *Umbellularia* and avocado are in the same family, Lauraceae.

In the avocado, infection occurred at various places on the leaf and appeared to be through the stomata, rather than following wounding. The spots were somewhat smaller and less angular than on *U. californica*. Reisolation of the organism from the avocado spots yielded the same organism with which the plant was originally inoculated.

Since the leaves of *U. californica* remain on the tree for several years, the pathogen is probably held over from season to season in infected leaves. The life cycle of this organism, therefore, appears to be relatively simple, the bacterium passing from old infected leaves to younger ones, dissemination probably being by rain, and penetration being chiefly through wounds caused by wind action or insects.

Classification

This organism appears to differ in a number of ways from bacterial organisms previously described and it does not key out to any known species of the Schizomycetes as listed in Bergey's Manual (1948). The organism isolated from *U. californica* is, therefore, proposed as a new species:

Pseudomonas lauracearum sp. nov. A motile rod with rounded ends and polar flagella; single, in pairs, or chains; average measurement 1.8 — 6.4 × 0.7 — 0.8 microns; non spore forming; aerobic; Gram-negative, non-acid-fast. On potato dextrose agar colonies white, butyrous, granular, flat, irregular in shape with lobate margins and opalescent luster; on beef peptone agar growth filiform, colonies cream colored, medium unchanged; liquification of gelatin slow and of the stratiform type; white pellicle formed on nutrient broth and on potato dextrose broth; no growth in Cohn's solution nor on Fermi's medium, but moderate growth in Uschinsk's

solution; growth retarded on culture medium containing 2½ per cent sodium chloride, no growth on culture medium containing 5 per cent sodium chloride; vitality on most culture media short. Acid, but no gas produced with glucose, sucrose, d-mannose, d-galactose and d-mannitol; no acid or gas produced with fructose, lactose, and dextrin. Starch not hydrolized, indol and hydrogen sulphide not produced; nitrates reduced to nitrites and nitrites to ammonia. Maximum temperature for growth 31° C., minimum 7° C. and optimum 22° C. Maximum pH for growth 9.2, minimum 5.4 and optimum 6.03. Pathogenic on *Umbellularia californica* Nutt. causing angular leaf spots; also pathogenic on leaves of avocado, *Persea drymifolia* Cham. and Schlecht. following inoculation. Specific name refers to the fact that both host plants belong to the family Lauraceae. Culture deposited with the American Type Culture Collection.

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Literature Cited

Bergey, D. H. et al. 1948. Manual of determinative bacteriology. xvi + 1529 pp. Wilkins Co. Baltimore, Md.
Elliott, C. 1920. Halo blight of oats. Jour. Agr. Res. 19: 139-172.
Johnson, J. 1947. Water congestion in plants in relation to disease. Univ. of Wisc. Res. Bull. 160. 35 pp.

HOWELLANTHUS, A NEW SUBGENUS OF PHACELIA

Lincoln Constance

Phacelia Dalesiana J. T. Howell, published (1937) under the title, "A Remarkable New Phacelia," fully lives up to the descriptive adjective. Although the author referred this northern California species to section *Euphacelia* "on characters of flowers and ovules," it was clear to him at the time that it was unique in many respects, and he has assured me that only its apparent similarity to "some otherwise entirely unrelated Mexican species" prevented him from describing it as a monotypic new genus. The possession of true interstaminal corolla scales and paired ovules afford it technical admission to *Euphacelia,* but it comprises a markedly discordant element in that section.

The writer's recent study of the subgenus *Cosmanthus* (1949) has provided data to permit comparison with the Mexican species mentioned above, and the cytological data accumulated by Cave and Constance (1942, 1944, 1947, 1950) afford a basis for contrasting *Phacelia Dalesiana* with other species and genera of Hydrophyllaceae in respect to chromosome number.